## II. REMARKS

Claims 2, 5-7, 17-19, and 21-23 are pending in the above-identified application. Claims 2, 5-7, 17-19 and 21 to 23 were examined and finally rejected.

No claims have been amended. Applicants respectfully request reconsideration and withdrawal of the outstanding rejections.

### 35 U.S.C. § 103

Claims 2, 5-7, 17-19 and 21-23 remain rejected under 35 U.S.C. § 103 (a) as allegedly unpatentable over either Li et al. or Rothwarf et al. (Reference C27 of Applicant's PTO-1449) in view of Traincard et al. and Epinat et al.

Briefly, and without repeating the stated grounds for rejection, the Office argued that the cited art would have led a skilled artisan to reasonably expect that since yeast do not endogenously produce the IKK proteins and contain no homologs of the NF-□B signaling system, a homogenous IKK complex formed from only the heterologously introduced IKK genes would have been expected, as now claimed by Applicants.

The Office argued that Traincard et al. teach that within eukaryotic systems, no homologs of any of member of the NF-kappaB signaling system (clearly disclosed as including Rel/NF-kappaB subunit genes and IKK gene) has been found within the genomes of *C. elegans* or *Saccharomyces cerevisia*, both of which were fully sequenced genomes at the time of the publication of Traincard et al. Epinat et al. is alleged to teach that yeast is a convenient host for the reconstitution of the NF-kappaB system since it does not contain an endogenous NF-kappB activity and that the reconstituted system provide an easy assay for testing stimuli or specific proteins that are postulated to be involved in NF-kappa signaling. Epinat et al. is alleged to show that an expressed I$\kappa$B protein could not be phosphorylated in yeast even under similar stimuli to those known to induce I$\kappa$B phosphorylation in mammals.

The Office also argued that Li et al. and Rothewarf et al. teach the importance of phosphorylation of the IKK complex for its kinase activity, teach that unstimulated cell

producing the IKK complex still have a basal level of kinase activity and further teach that the IKK complex can be phosphorylated in vitro by the NIK and MEKK1 proteins to produce an active complex. As such, the Office alleged that one of skill in the art would reasonably expect that coexpression of the three subunits together in yeast would produce a complex that would have the basal level of kinase activity demonstrated by the unstimulated cells of Li et al. and Rotherwarf et al. Additionally, the Office alleged that even if this in fact proved not to be the case, a skilled artisan would have clearly expected that active complex could be produced by coexpression of either NIK or MEKK1 in the yeast host, which is allegedly clearly taught by the art.

Applicants respectfully traverse the rejection for the reasons which follow.

Applicants provide with this response a Declaration under 37 C.F.R. § 1.131 by Ebrahim Zandi and Beth Schomer Miller, co-inventors of the subject application. The Declaration establishes the conception and reduction to practice in the United States the transformation of an IKK subunit gamma ($\gamma$) gene, an IKK subunit alpha ($\alpha$) and/or an IKK subunit beta ($\beta$) gene into yeast and the separation from that yeast a substantially homogenous and biologically functional IKK protein complex prior to November 15, 2000, the online publication date of the literature article Li et al. (2001) "Role of IKK$\gamma$/NEMO in Assembly of the I$\kappa$B Kinase Complex" Journal of Biological Chemistry 276(6):4494-4500. In view of this Declaration, Li et al. is not prior art to the present claims. Therefore, Applicants respectfully request removal of the Li et al. reference.

Accordingly, in an effort to further prosecution, Applicants address the rejection under 35 U.S.C. § 103(a) using the application of Rothwarf et al. in view of Traincard et al. and Epinat et al.

Applicants agree that the cited prior art (namely Traincard et al. and Epinat et al.) teach that no member of the NF-kappaB pathway exists in *Saccharomyces cerevisia* at the time of the application and that yeast lacked the ability to phosphorylate the I$\kappa$B protein. However, in comparing the teachings of the references in combination,

Applicants note that they do not teach or suggest that the production of substantially homogenous and biologically functional IKK protein complex because Rothwarf et al.: 1) does not teach the autophosphorylation of the IKK complex by the IKK$\gamma$ subunit; 2) does teach that the mammalian IKK complex requires post-translational processing or "activation" by protein kinases to produce biologically functional IKK complex in mammalian cells and 3) does not teach that the active IKK complex produced in yeast could be produced by coexpression of either NIK or MEKK1.

Applicants provide with this response a Declaration under 37 C.F.R. § 1.132 by Ebrahim Zandi, Ph.D. As stated by Dr. Zandi:

> "Rothwarf et al., supra, stated on page 300, left column, first paragraph 'The ability of the C-terminally truncated IKK-$\gamma$ mutant to inhibit IKK activation by upstream stimuli, while having only a small effect on basal kinase activity, indicates that the major function of IKK-$\gamma$ may be to connect the IKK complex to upstream activators.'"

Dr. Zandi also states that in the context of the entire article, this statement teaches away from the autophosphorylation activity of the IKK$\gamma$ subunit shown in the above-identified application in Example II, first paragraph, "IKK$\gamma$ regulates the autophosphorylation of the T loop residues in the kinases domain of the IKK$\beta$. This phosphorylation is required for activation of the IKK complex." Thus, contrary to the Office's position, it would not have been obvious to one of ordinary skill in the art to prepare substantially homogenous and biologically functional IKK protein complex in a yeast system as the autophosphorylation of the IKK complex by the IKK$\gamma$ subunit, which the application shows is required for activation of the IKK complex, was unknown in the prior art

Furthermore, as stated by Dr. Zandi, at the time of the publication of the article, upstream activating proteins were understood by the authors of Rothwarf et al. to be required to activate IKK complex. The data described in Figure 3 and on page 15, lines 21 to 27 in the above-identified application show that the activity of recombinantly produced IKK complex isolated from yeast is higher than the purified IKK complex from

non-stimulated HeLa cells and the same or slightly higher than purified activated IKK complex from TNF-stimulated HeLa cells. In Applicant's subsequent experiments, described in Miller and Zandi (2001) "Complete Reconstitution of Human IκB Kinase (IKK) Complex in Yeast" J. Biol. Chem. 276(39):36320-36326 (enclosed as Appendix B of the enclosed Declaration under 37 C.F.R. § 1.132), Figure 3A, 3B and left column, lines 14-20, the activity of recombinantly produced IKK complex isolated from yeast is shown to be intermediate to the purified IKK complex from non-stimulated and TNF-stimulated HeLa Cells. In sum, the activity of recombinantly produced IKK complex isolated from yeast is higher than the purified IKK complex from non-stimulated HeLa cells. Thus, production of biologically functional IKK protein complex produced by the yeast system was surprising and unexpected. Dr. Zandi states it was believed that mammalian IKK complex required post-translational processing or "activation" by protein kinases to produce biologically functional IKK complex in mammalian cells. Yeast were not known to possess the identical protein kinases or other homologous kinases to the mammalian "activating" proteins, such as TRAF2, RIP, and A20. This is supported by the authors of the following technical publications: Devin et al. (2000) Immunity **12**:419-429; Zhang et al. (2000) Immunity **12**:301-311; and Lin et al. (2000) Mol. Cellular Biol. **20**(18):6638-66445, Appendices B, C and D of the enclosed Declaration under 37 C.F.R. § 1.132.

As described by Dr. Zandi, Devin et al., *supra*, discloses that the RIP kinase and the TRAF2 protein are essential effectors in the TNF signaling pathway mammalian cell systems. In response to TNF treatment, the transcription factor NF-κB is activated through activated IKK. Moreover, the reference discloses that IKK activation requires the presence of RIP in the same complex (see Summary and Introduction). Zhang et al. (2000), *supra*, also discloses that the signaling activation of the IKK signalsome are regulated through binding of NEMO (IKKγ) to RIP and A20 through the p55 TNF receptor complex (see Summary and Introduction). Lin et al., *supra*, also discloses that the death domain kinase RIP, a key factor in TNF signaling, plays a pivotal role in TRAIL-induced IKK and JNK activation. Thus, one of skill in the art would not have

expected that yeast could produce biologically functional IKK complex because yeast lack all of these necessary activating proteins to produce such a biologically functional IKK complex.

The Office also alleged that one of skill in the art would have "... clearly expected that active complex could be produced by coexpression of either of NIK or MEKK1 in the yeast host as this is clearly taught by the art." As stated by Dr. Zandi,

> "Rothwarf et al., *supra*, on page 297, right column, lines 16 – 19, teaches that IKK-$\alpha/\beta$ can be phosphorylated and activated by overexpression of NIK and MEKK1 in mammalian cells, but does not teach that the IKK complex from yeast of the present application containing IKK$\alpha$, IKK$\beta$, and IKK$\gamma$ can be activated by NIK or MEKK1 in yeast systems. Rothwarf et al. also teaches that the physiological role of NIK and MEKK1 in IKK activation by pro-inflammatory cytokines is not clear."

Thus, as stated by Dr. Zandi, this statement shows that the authors were uncertain as to the role of NIK or MEKK1 in activating IKK proteins. Therefore, Rothwarf et al. does not teach nor would one of ordinary skill in the art expect that the active IKK complex produced in yeast could be produced by coexpression of either NIK or MEKK1.

Applicants respectfully request reconsideration that, as of the effective filing date, one of skill in the art would not have expected that yeast would be capable of producing substantially homogenous and biologically functional IKK complex because of the arguments presented above. Accordingly, because the prior art as a whole taught against the claimed invention and thus, an expectation of success was lacking, Applicants submit that the rejection is in error and respectfully request its withdrawal.

**Supplemental Information Disclosure Statement**

Attached to this Response is a Supplemental Information Disclosure Statement for consideration and entry into the file.

## III. CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

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